

To the claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-46 (Cancelled)

Claim 47 (Original): A method of generating a population of rAAV particles comprising culturing a producer cell under a stress condition, said producer cell comprising (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) helper virus function for AAV, whereby about two-fold or more rAAV particles are produced compared to a producer cell not grown under said stress condition.

Claim 48 (Original): The method of claim 47, wherein the producer cell is attachment dependent.

Claim 49 (Currently amended): The method of ~~claim~~ claim 47, wherein the producer cell is grown in suspension.

Claims 50-92 (Cancelled)

Claim 93 (Currently amended): A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

- a) ~~providing an AAV producer cell that comprises:~~
 - ~~(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;~~
 - ~~(ii) a recombinant AAV (rAAV) pro-vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and~~
 - ~~(iii) a helper virus for AAV or a polynucleotide sequence of said helper virus that encodes at least one helper virus function;~~

b) ~~a)~~ subjecting the ~~an~~ AAV producer cell ~~provided in step a)~~ to a sub-lethal stress condition; wherein said AAV producer cell comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) pro-vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) a helper virus for AAV or a polynucleotide sequence of said helper virus that encodes at least one helper virus function; and

e) ~~b)~~ incubating the stressed producer cell of step b) ~~a)~~ under conditions that are permissive for replication of AAV;

whereby rAAV particles are produced.

Claim 94 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is selected from the group consisting of a nutritional stress, an osmotic stress, a pH stress, a temperature stress, an aerobic stress, a mechanical stress, a radiational stress and a toxic stress.

Claim 95 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is a nutritional stress.

Claim 96 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is an osmotic stress.

Claim 97 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is a pH stress.

Claim 98 (Original): A method of generating a population of rAAV particles according to claim 97, wherein said pH stress comprises raising the pH to above pH 7.2.

Claim 99 (Original): A method of generating a population of rAAV particles according to claim 97, wherein said pH stress comprises elevating the pH to at least 7.4, and wherein the majority of the AAV particles produced are released into the supernatant.

Claim 100 (Original): A method of generating a population of rAAV particles according to claim 97, wherein said pH stress comprises elevating the pH to about 8.0.

Claim 101 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is a temperature stress.

Claim 102 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is an aerobic stress.

Claim 103 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is a mechanical stress.

Claim 104 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is a radiational stress.

Claim 105 (Currently amended): A method of generating a population of rAAV particles according to claim 93, wherein said ~~sub-lethal~~ stress is a toxic stress.

Claim 106 (Original): A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in one or more amino acids.

Claim 107 (Original): A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in aspartic acid.

Claim 108 (Original): A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in glutamic acid.

Claim 109 (Original): A method of generating a population of rAAV particles according to claim 108, wherein the deficient medium contains less than 10 $\mu\text{mol/L}$ of aspartic acid.

Claim 110 (Original): A method of generating a population of rAAV particles according to claim 95, wherein the deficient medium contains less than 2 $\mu\text{mol/L}$ of glutamic acid.

Claim 111 (Original): A method of generating a population of rAAV particles according to claim 95, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in serum.

Claim 112 (Original): A method of generating a population of rAAV particles according to claim 95, wherein the cells are subjected to said nutritional stress by introducing the cells into a nutritionally deficient medium.

Claim 113 (Original): A method of generating a population of rAAV particles according to claim 95, wherein the cells are subjected to said nutritional stress by culturing the cells in a medium until the medium becomes nutritionally deficient.

Claim 114 (Currently amended): A method of generating a population of rAAV particles according to claim 93 117, wherein said purified population of rAAV vector particles is substantially free of replication-competent AAV and of helper virus and cellular proteins.

Claim 115 (Currently amended): A method of generating a population of rAAV particles according to claim 93 118, in which elution from the chromatographic resin is conducted by increasing the salt concentration and chromatographic eluants comprising rAAV particles are subsequently treated to reduce the effective salt concentration by dilution, dialysis, diafiltration or concentration.

Claim 116 (Currently amended): A method of generating a population of rAAV particles according claim 93 118, including the step of subjecting a fraction comprising AAV particles to heparin sulfate chromatography.

Claim 117 (Currently amended): A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

- a) ~~providing an AAV producer cell that comprises:~~
 - (i) ~~one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;~~

~~(ii) a recombinant AAV (rAAV) pro-vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and~~

~~(iii) a helper virus for AAV;~~

b) a) incubating the an AAV producer cell provided in step a) under conditions that are permissive for replication of AAV and which comprise inducing a sub-lethal stress condition in the AAV producer cell; wherein said AAV producer cell comprises:

(i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein;

(ii) a recombinant AAV (rAAV) pro-vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and

(iii) a helper virus for AAV or a polynucleotide sequence of said helper virus that encodes at least one helper virus function;

e) b) lysing the producer cell after the incubation of step b) a) to produce an AAV producer cell lysate; and

d) c) purifying the AAV producer cell lysate to generate a population of recombinant adeno-associated virus (rAAV) particles.

Claim 118 (Currently amended): A method of generating a population of rAAV particles according to claim 117, wherein said purifying step d) c) comprises chromatographing the AAV producer cell lysate of step e) b) on at least one positively-charged anion exchange resin followed by purifying on either a cation exchange resin or by tangential flow filtration to generate a purified population of rAAV vector particles.

Claim 119 (Currently amended): The method of claim ~~118~~ 117, wherein said purifying step d) c) comprises chromatographing the AAV producer cell lysate of step e) b) on at least one negatively-charged cation exchange resin followed by purifying on an anion exchange resin.

Claim 120 (Currently amended): A method of generating a population of rAAV particles according to claim 117, wherein said purifying step d) c) comprises chromatographing the AAV producer cell lysate of step e) b) on a positively-charged anion exchange resin followed by tangential flow filtration to generate a purified population of rAAV vector particles.

Claim 121 (Original): A method of generating a population of rAAV particles according to claim 117, wherein said rAAV pro-vector comprises a heterologous non-AAV polynucleotide flanked by two AAV inverted terminal repeats (ITRs).

Claim 122 (Original): A method of generating a population of rAAV particles according to claim 117, wherein said AAV producer cell comprises at least one AAV packaging gene that is stably integrated into the genome of said AAV producer cell.

Claim 123 (Original): A method of generating a population of rAAV particles according to claim 117, wherein said AAV producer cell comprises an AAV *rep* gene and an AAV *cap* gene.

Claim 124 (Original): A method of generating a population of rAAV particles according to claim 117, wherein said helper virus is adenovirus.

Claims 125-177 (Cancelled)

Claim 178 (New): A method of generating a population of rAAV particles according to claim 47, whereby about five-fold or more rAAV particles are produced compared to a producer cell not grown under said stress condition.

Claim 179 (New): A method of generating a population of rAAV particles according to claim 47, whereby about ten-fold or more rAAV particles are produced compared to a producer cell not grown under said stress condition.

Claim 180 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is selected from the group consisting of a nutritional stress, an osmotic stress, a pH stress, a temperature stress, an aerobic stress, a mechanical stress, a radiational stress and a toxic stress.

Claim 181 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is a nutritional stress.

Claim 182 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is an osmotic stress.

Claim 183 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is a pH stress.

Claim 184 (New): A method of generating a population of rAAV particles according to claim 183, wherein said pH stress comprises raising the pH to above pH 7.2.

Claim 185 (New): A method of generating a population of rAAV particles according to claim 183, wherein said pH stress comprises elevating the pH to at least 7.4, and wherein the majority of the AAV particles produced are released into the supernatant.

Claim 186 (New): A method of generating a population of rAAV particles according to claim 183, wherein said pH stress comprises elevating the pH to about 8.0.

Claim 187 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is a temperature stress.

Claim 188 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is an aerobic stress.

Claim 189 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is a mechanical stress.

Claim 190 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is a radiational stress.

Claim 191 (New): A method of generating a population of rAAV particles according to claim 47, wherein said stress is a toxic stress.

Claim 192 (New): A method of generating a population of rAAV particles according to claim 181, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in one or more amino acids.

Claim 193 (New): A method of generating a population of rAAV particles according to claim 181, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in aspartic acid.

Claim 194 (New): A method of generating a population of rAAV particles according to claim 181, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in glutamic acid.

Claim 195 (New): A method of generating a population of rAAV particles according to claim 194, wherein the deficient medium contains less than 10 $\mu\text{mol/L}$ of aspartic acid.

Claim 196 (New): A method of generating a population of rAAV particles according to claim 181, wherein the deficient medium contains less than 2 $\mu\text{mol/L}$ of glutamic acid.

Claim 197 (New): A method of generating a population of rAAV particles according to claim 181, wherein said nutritional stress is imposed by culturing the producer cells in a medium that is deficient in serum.

Claim 198 (New): A method of generating a population of rAAV particles according to claim 181, wherein the cells are subjected to said nutritional stress by introducing the cells into a nutritionally deficient medium.

Claim 199 (New): A method of generating a population of rAAV particles according to claim 181, wherein the cells are subjected to said nutritional stress by culturing the cells in a medium until the medium becomes nutritionally deficient.